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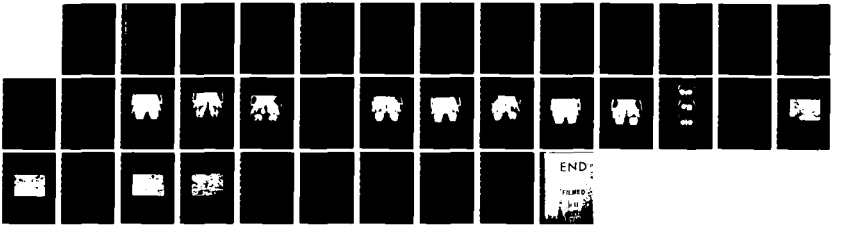

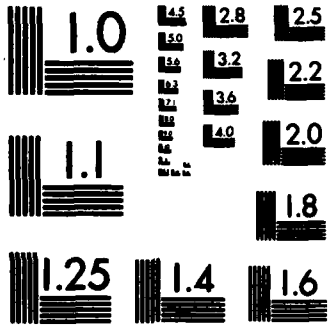

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REPORT NUMBER 7

MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS AND MANAGEMENT OF OROFACIAL FRACTURES

ANNUAL REPORT

Craig R. Hassler and Larry G. McCoy

May, 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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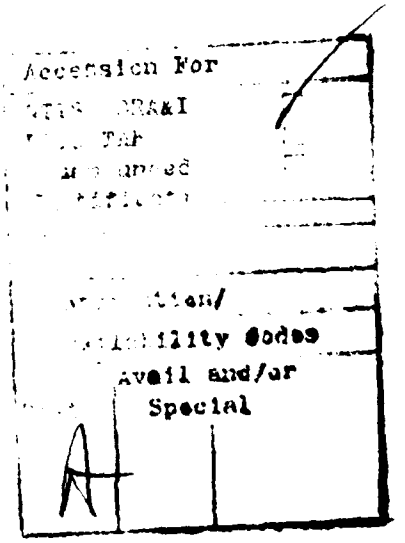

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Research studies were continued to further our understanding of the in vivo behavior of resorbable calcium phosphate ceramics for use in the management of hard tissue avulsive wounds and orofacial fractures.

Material processing studies were conducted to develop porous tricalcium phosphate materials of directional porosity. An observation of the critical importance of pore directionality was made and reported in the last

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annual report. Consequently, a material with directional porosity was envisioned which would allow adequate ingrowth of bone through the biomaterial prior to loss of mechanical integrity of the biomaterial. Classically, porous biodegradable biomaterial designs have had omnidirectional pores which limit the strength of the biomaterial. A controlled unidirectional material is free from the above design constraint. Numerous possibilities are thus available.

The overall objective is to produce a completely resorbable biomaterial which will promote bone formation and after the bone remodeling, biodegradation process be completely replaced by bone. It should be pointed out that the dynamics of this situation are complex. The biomaterial should allow bone ingrowth and provide mechanical integrity during the remodeling, biodegradation process. The simultaneous dissolution of the biomaterial and bone formation need to proceed in a parallel and controlled fashion, so that mechanical integrity of the area under repair is not lost. In previous studies, omnidirectional structural material would, depending upon chemical composition, either: not entirely biodegrade, or would degrade until mechanical integrity was lost. The specific objective of this study was to find a method of producing unidirectional porosity tricalcium phosphate material for large segment repair and then test the material in vivo.

Numerous attempts were made to prepare a biomaterial with unidirectional porosity. Early attempts failed because these materials were too fragile along the axis of the pores to be of any practical value. Later efforts were hampered by destruction of the samples during sintering. The high volume of gas created by decomposition of the polymer void former used was responsible for the destruction. Finally, an organic (silk) void former was utilized, and unidirectional samples were prepared with 300 micron pores. These samples were less than ideal in pore spacing and strength, however, the material was judged adequate for proof of concept studies.

Large segment samples were supplied to USAIDR for implantation in dog mandibles. The same material was implanted into the calvaria of rabbits at Battelle-Columbus Laboratories. Implants approximately 8 mm in diameter were placed bilaterally in the calvaria of the animals and were evaluated for periods of 3, 6, 9, and 12 months. At each time period, a portion of the animal population was necropsied and analyzed by histologic and radiographic methods. The results of the in vivo study indicate that unidirectional porosity will allow bone ingrowth and biodegradation without loss of implant integrity. This finding is in marked contrast to the previously reported findings with omnidirectional porosity material. Striking examples of improved bone ingrowth and maintenance of strength of the formed bone matrix were found when the pore orientation was in the direction of intended bone growth. That is, from the fresh bone site or fresh cut of bone transversely through the implant material. The analysis suggests that the concept of large diameter porosities, oriented in the direction of desired bone growth, is feasible. These results were corroborated by Tortorelli at USAIDR. However, a higher strength material with a higher density of unidirectional pores is desirable.

MANAGEMENT OF HARD TISSUE AVULSIVE WOUNDS AND MANAGEMENT OF OROFACIAL FRACTURES

by

Craig R. Hassler and Larry G. McCoy

SUMMARY

Research studies were continued to further our understanding of the *in vivo* behavior of resorbable calcium phosphate ceramics for use in the management of hard tissue avulsive wounds and orofacial fractures.

Material processing studies were conducted to develop porous tricalcium phosphate materials of directional porosity. An observation of the critical importance of pore directionality was made and reported in the last annual report. Consequently, a material with directional porosity was envisioned which would allow adequate ingrowth of bone through the biomaterial prior to loss of mechanical integrity of the biomaterial. Classically, porous biodegradable biomaterial designs have had omnidirectional pores which limit the strength of the biomaterial. A controlled unidirectional material is free from the above design constraint. Numerous possibilities are thus available.

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FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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BACKGROUND, PROBLEM AND APPROACH

Historically, various techniques have been employed for the repair or treatment of osseous diseases, defects, or wounds. Autogeneous bone grafting remains the most satisfactory approach, but is not without the disadvantages associated with double surgeries, limits in structural properties, and the limitations imposed on the repair of massive osseous defects.

Since April, 1970, Battelle's Columbus Laboratories has been conducting research under contract with the Dental Research Division, U.S. Army Medical Research and Development Command, on the development of resorbable ceramics for potential application in the repair of hard tissue avulsive wounds. The basic materials have been calcium phosphates. These materials were selected because they contain two of the essential elements of the natural bone mineral phase, calcium hydroxyapatite.

In vivo studies were conducted initially at the U.S. Army Institute of Dental Research (USAIDR), using the sintered porous materials and slurries prepared at Battelle from tricalcium phosphate $Ca_3(PO_4)_2$ and other calcium orthophosphate powders $CaHPO_4$ and $Ca(H_2PO_4)_2$, to evaluate the potential use of calcium phosphates to both facilitate repair of bone defects and to determine the best material for future exploration[1-3]. The implant studies indicated that calcium phosphates consisting essentially of the mineral phases $Ca(PO_3)_2$, $Ca_3(PO_4)_2$, and $CaHPO_4$ are well tolerated by the tissue, appear to be nontoxic, are resorbable, and permit rapid invasion of new bone.

Of the various porous calcium phosphate materials investigated, tricalcium phosphate, $Ca_3(PO_4)_2$, was selected for continued development and evaluation since it was easy to fabricate and was found to be both biocompatible and resorbable. Emphasis has been directed toward producing porous materials consisting of single-phase tricalcium phosphate[4-7]. Research on granular formations of tricalcium phosphates (TCP) continued at USAIDR. Basic research at Battelle-Columbus was focused on producing practical large segment replacement implants from TCP.

To provide basic resorption rate data on the in vivo behavior of solid tricalcium phosphate bioresorbable ceramics, implant studies were initiated in 1975 at Battelle-Columbus using the rabbit calvarium model[8]. Early samples of tricalcium phosphate were implanted as a control and samples of two new materials were implanted for comparative observation. These new materials were prepared using the improved processing techniques derived in previous materials development studies and represented significant improvements in the structural characteristics of porous tricalcium phosphate. The characterization of the materials involved and the results of the in vivo studies were the subject of the Fifth Report[8].

These results indicated that the improved material exhibited significant increases in resorption rate. In fact, the material resorbed so rapidly that after the ninth month the implant appeared to be granulated and was invaded with connective tissue. This result does not imply lack of biocompatibility, but does suggest that such rapid degradation can be deleterious in stress-bearing situations. It was not known then whether the enhanced resorptivity resulted from achieving a Ca/P ratio closer to the theoretical for tricalcium phosphate or from the improvements in the structural characteristics of the material.

To discern the effects of structural variations on resorption rate, experimental porous implants were prepared using a single tricalcium phosphate powder with different pore size distribution. Three materials were prepared for in vivo evaluation. These studies demonstrated that orientation of pore structure is a more important variable than pore size distribution.[9] Assuring interconnection of porosity was indicated as another problem which could probably be overcome by a redesign of the pore forming technique. The study indicated that a higher density material of the stoichiometric chemistry with directional porosity is probably the desired material.

MATERIALS AND METHODS

Porous Materials Development

The following work has been completed in the production of first generation directed porosity biomaterials.

Certified ACS calcium carbonate and phosphoric acid (assay 85.4 percent) were slowly mixed in distilled water at 180 degrees Fahrenheit to form a tricalcium phosphate (TCP) slurry. The slurry was air dried, then dried under vacuum at 220 degrees Fahrenheit overnight to produce the tricalcium phosphate powder. This powder was then used in fabricating the directed porosity blocks.

Initial experiments were directed at fabricating a unidirectional porosity tricalcium phosphate block with a density greater than 90 percent of the calculated theoretical density. Blocks were formed in a steel die by alternately layering the tricalcium phosphate powder and the rows of pore forming fibers until a suitable thickness was obtained. The blocks were pressed at 25,000 psi and fired. Upon firing, the fibers (sutures in the initial experiments) were burned out forming the pores. The fired compacts fractured easily, parallel to the direction of the pores, possibly due to residual stresses in the material.

To alleviate this fracture problem pore formers differing chemically and physically were tried. New tricalcium phosphate samples were made by cold pressing loose powder around 2 to 3 layers of nylon or polypropylene mesh screen material. The screen was a clean-burning, void-forming material. However, upon burn out (firing) large volumes of gases were released. This outgassing caused delamination of the blocks. A slower burn out rate was tried, but did not eliminate this problem.

A third method was then attempted. It was found that by using a No. 8 size silk thread (~300 micron) unidirectional pores could be formed in tricalcium phosphate blocks. This procedure consisted of layering TCP powder 1/8 inch thick in a steel die, and adding parallel rows 1/8 inch apart of wax coated silk thread, followed by another layer of powder. This process was repeated to produce 8 layer silk thread compacts. The compacts were fired to

500° C incrementally over a five day period to burn out the silk threads. The compacts were then sintered for two hours at 1150°C to produce a unidirectional tricalcium phosphate block.

The material manufactured by this procedure was judged adequate for proof of concept in vivo analysis. Unfortunately, the material was much weaker than desired, the pore density was much lower than desired, and the pores were only unidirectional, and not interconnected.

EXPERIMENTAL ANIMAL STUDIES

This portion of the report details the various research procedures which are used in our laboratories to evaluate biodegradable materials. The procedures include histology, radiography, and quantitative histology. The classical techniques of histology and radiography are the key diagnostic procedures.

Research Protocol

In order to test the biodegradation of large tricalcium phosphate segments, a special experimental model has been devised in this laboratory. We utilize the calvarium of a mature, male New Zealand White rabbit with a minimum weight of 8 pounds. The calvarium has been found to be an excellent implant site for this relatively weak structural biomaterial since stresses upon the calvarium are not extraordinarily high, therefore, external stabilization is not required. Consequently, confusing effects which might be due to stabilization devices are not seen. Of greater importance is the fact that this implant site provides the researcher with a large, relatively uniform area for various simultaneous studies such as periodic radiography, multiple histologic analyses, etc.

Standard aseptic surgical technique was used to expose the calvarium of the anesthetized animal. A circular, 8 mm diameter, portion of the calvarium was osteotomized bilaterally from the animal with no attempt to salvage the periosteum overlying the excised area. The tricalcium phosphate implants, were aligned with directional porosity parallel to the animals' long axis and interference fit. The skin incision was closed and the animal was

treated with a prophylactic antibiotic. Eight animals were randomly separated into four experimental groups. The experimental groups consisted of sacrifice dates, 3, 6, 9, and 12 months post surgery. Since two implants were placed in each animal, four samples were available for analysis at each sacrifice interval.

The animals were radiographed at 3-month intervals until the time of necropsy and the excised skulls were radiographed post-necropsy. The histologic technique consisted of embedding portions of the excised calvarium-tricalcium phosphate complex in methyl methacrylate and sectioning. Half of each excised sample was stained with basic fuchsin prior to sectioning. During the experiment, rabbits were stained at time zero and 3-month intervals with one of the following vital bone growth markers: tetracycline 60 mg/kg, DCAF 20 mg/kg and xylenol orange 90 mg/kg. The other half of the sample was left unstained and sectioned for ultraviolet bone growth analysis utilizing these previously injected vital bone growth markers.

Radiographic Examination of Tricalcium Phosphate Biodegradability

Radiographs of the rabbits were taken at time zero, 3-month intervals and of the excised skull after necropsy to monitor the biodegradation of the tricalcium phosphate implant. These high resolution radiographs were obtained using fine-grained industrial X-ray film and a Picker Industrial X-Ray Unit.

Representative of the results are the radiographs of rabbit E-79. Figure 1 shows *in situ* tricalcium phosphate implants four days post surgery. Note that the implants are readily apparent in the animals' calvarium. In this "live" X-ray detail of the granular nature of the implant is not distinct. The radiodensity of both implants appears equivalent.

Figure 2 is a radiograph of the same animal (E-79) 3 months post implant. Note that some mottled areas of lower radiodensity are apparent within the samples. These areas suggest biodegradation within the samples. There is also some evidence of degradation around the periphery of these circular samples.

By six months, there is a dramatic alteration in the appearance of the samples. Figure 3 illustrates the 6-month radiograph of rabbit E-79. The

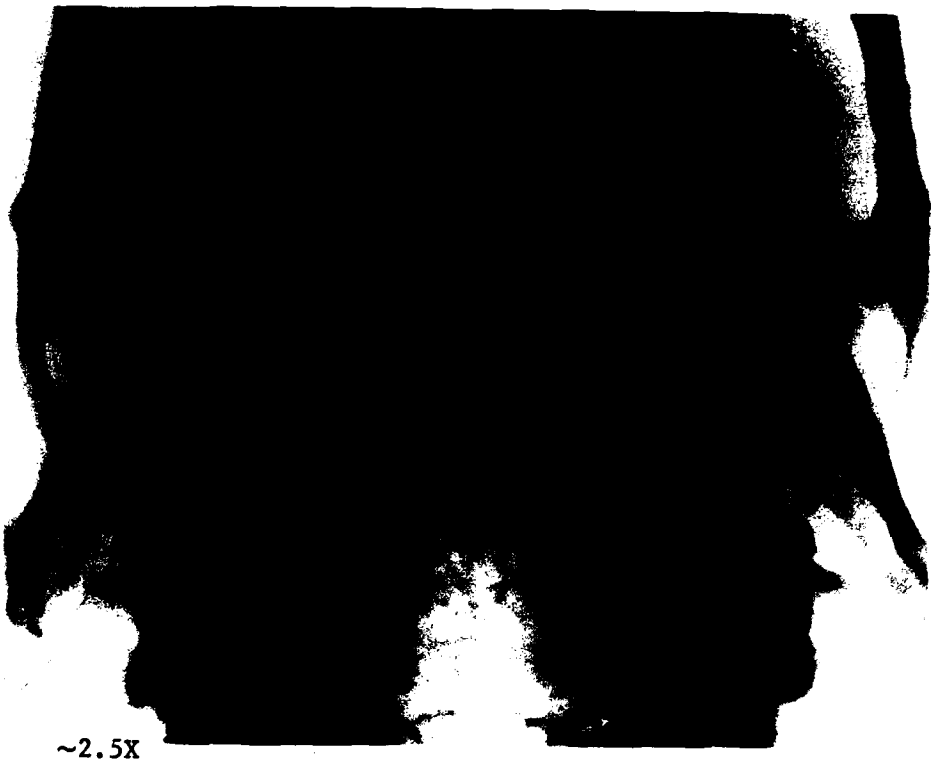

FIGURE 1. Radiograph of Rabbit E-79 at four days post surgery. This radiograph shows the two circular samples of tricalcium phosphate in the rabbits' calvarium. The granular appearance of the samples is due to the porosity of the specimens. The quality of the radiograph is limited by the interferring structures. The next three radiographs (Figures 2-4) will compare the changes in radiodensity of this particular sample with time.

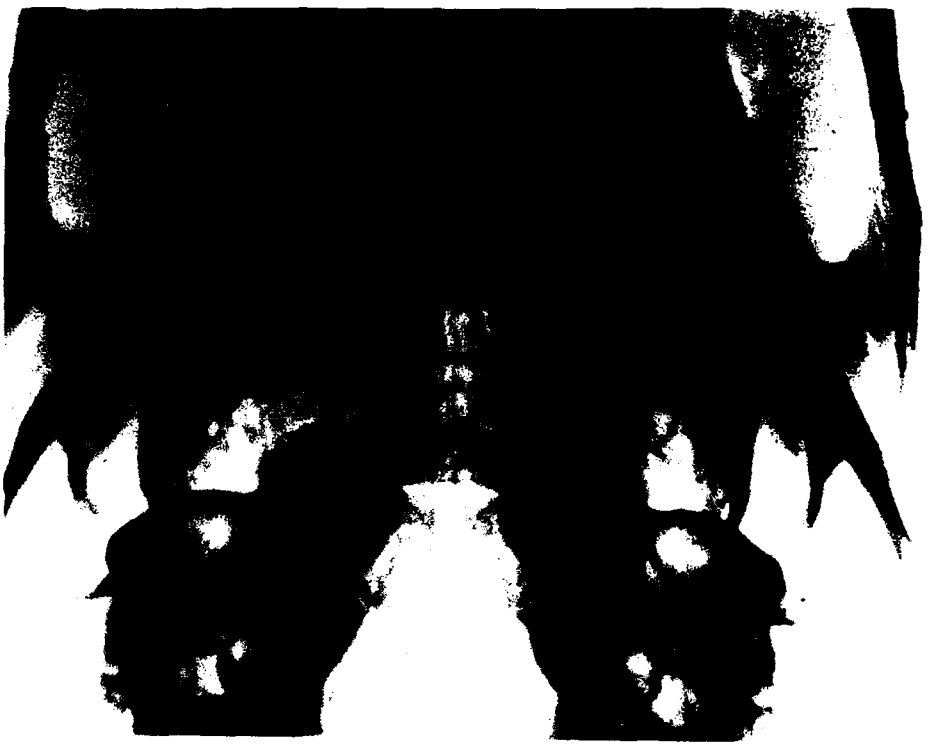

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FIGURE 2. Radiograph of Rabbit E-79 at three months post surgery. The samples have become somewhat less radiodense than at time of implant (Figure 1). This decrease of radiodensity is noted in the alteration of the mottled areas of the implants. A radiolucent area appears to be developing around the left implant.

~2.5X

FIGURE 3. Radiograph of Rabbit E-79 at six months post surgery. This radiograph demonstrates a dramatic decrease in radiodensity when compared to the previous two figures. This decrease in radiodensity is especially apparent when the right sample is compared to the previous two figures (Figures 1 and 2). This decrease in radiodensity implies bioresorption of the samples.

right sample demonstrates a dramatic decrease in radiodensity. This decrease in radiodensity is not as dramatic in the left sample. The left sample demonstrates some radiolucency at the periphery.

Figure 4 is the 12-month radiograph of rabbit E-79. The biodegradation of both samples appears to be continuing; however, the change in radiodensity is not as dramatic as that seen between 3 and 6 months. The left-right sample difference in radiodensity persists in that the bioresorption continues to be advanced in the right side sample.

The same alteration in radiodensity can also be seen in another 12-month experimental animal. Figure 5 (rabbit C-79) shows the zero time radiograph, taken 6 days post implant. Figure 6 shows the same samples 12 months post implant. The decrease in radiodensity is not as dramatic as the previous example; however, radiodensity has decreased, indicating bioresorption. It is important to note that no loss of implant-bone integrity is evident with these unidirectional porosity specimens. It should be recalled that deleterious integrity loss was noted radiographically in previous experiments where omnidirectional porosity rather than unidirectional was utilized.

Another example of bioresorption of the samples is illustrated by radiography from rabbit H-79 at time zero (one day post surgery) in Figure 7, and nine months post surgery in Figure 8. In this case, the decrease in radiodensity of the samples is quite dramatic, especially in the right-hand sample.

The initial fit of the left sample is poorer than the right, as indicated by radiolucency in Figure 7. These gaps are corroborated by the surgical notes. Surprisingly, these radiolucencies persist in the 9 month radiograph (Figure 8). Necropsy notes report that this left sample was rigid at necropsy.

Another indication of the amount of resorption which has occurred can be seen in Figure 9. This figure is a collage of post-necropsy radiographs. For these radiographs the calvarium has been excised. Consequently, all interferring structures have been removed. Animal D-79 (Figure 9A) is a three-month necropsy. Animal H-79 (Figure 9B) is a nine-month necropsy and animal E-79 (Figure 9C) is a 12-month necropsy.

~2.5X

FIGURE 4. Radiogrpah of Rabbit E-79 at 12 months post surgery. This one-year radiograph shows the same pattern of progressive decrease in radiodensity as compared to the previous figures. The change in radiodensity from Figure 3 at 6 months is not as dramatic as that seen between month 3 and month 6 (Figure 2 vs. Figure 3). However, in the right hand sample the decrease is greater than in the left. This progressive decrease in radiodensity implies a progressive bioresorption.

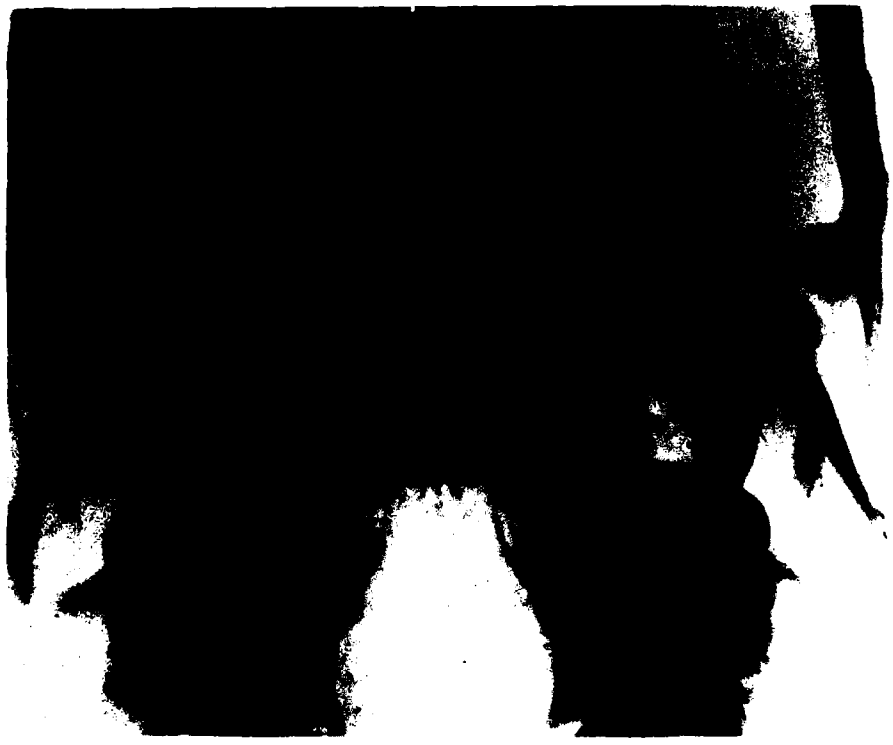

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FIGURE 5. Radiograph of Rabbit C-79 at 6 days post surgery. This radiograph is for comparison to Figure 6, where the same implants are seen 1 year post surgery.

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FIGURE 6. Radiograph of Rabbit C-79, one year post surgery. When compared to Figure 5 (the same animal post surgery) a definite decrease in radiodensity (indicating bioresorption) can be noted. The change is not as dramatic as that seen in the previous example (Rabbit E-79). This difference between the two rabbits probably reflects inconsistency in the samples. It is important to note that no loss of implant-bone integrity is seen.

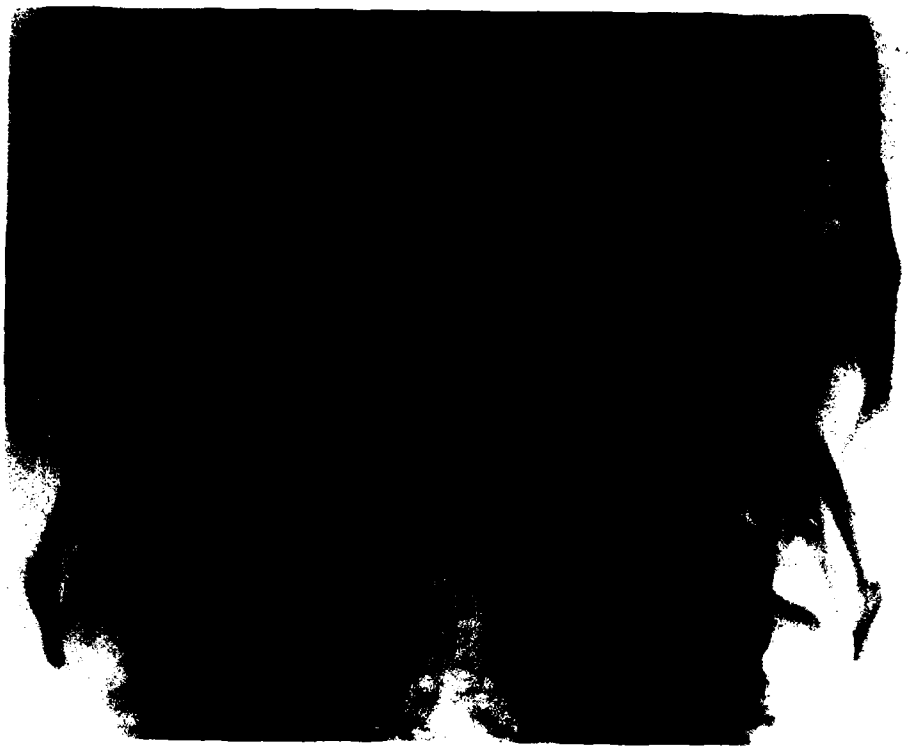

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FIGURE 7. Radiograph of Rabbit H-79, one day post surgery. This figure is for comparison to Figure 9. The two tricalcium phosphate samples can be clearly seen in the animal's calvarium. This radiograph is similar to the previous immediate post implant radiographs.

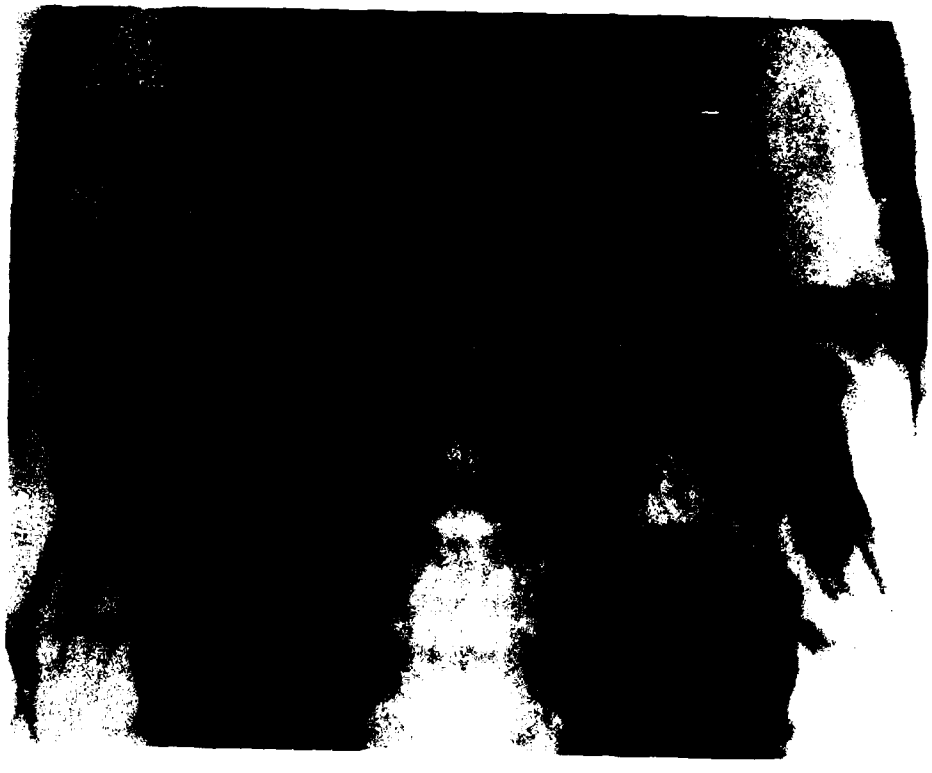

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FIGURE 8. Radiograph of Rabbit H-79 nine months post surgery. The radiodensity of the implants, expecially that on the right has decreased dramatically. This decrease in radiodensity implies significant bioresorption of the samples. As with the previous examples, there appears to be no loss of implant-bone integrity.

A

B

C

~2.5X

FIGURE 9. Collage of excised post necropsy radiographs. The radiographs are from excised calvaria of three different animals at: A, 3 months (Rabbit D-79), B, 9 months (Rabbit H-79) and C, 12 months (Rabbit E-79). A progressive decrease in radiodensity of the samples can be seen with time. Radiodensity changes are most dramatically illustrated in the 9 and 12 month samples (B and C). Bioresorption is most obvious about the periphery of the samples. However, progressive changes in radiodensity with time can also be seen through the center of the samples. Excised calvarium radiographs provide better definition due to the removal of interferring structures. Unfortunately the same animal cannot be progressively analyzed using this technique.

Changes in the bioresorption pattern can be seen especially about the periphery of the samples. Decreases in radiodensity are apparent, especially within the 9 and 12 month samples. There is a marked difference in the degree of resorption. For example, the right-hand sample from rabbit H-79 at 9 months appears to exhibit more resorption than samples seen in rabbit E-79 at 12 months. (Caution must be used in the interpretation of these radiographs.) Since these radiographs are from three different animals, inter-animal differences are difficult to account for. However, a general trend of increased resorption with time can be noted as with the previously exhibited "live" radiographs.

Histologic Evaluations

To evaluate the rate of ingrowth of biologic material (bone and connective tissue) into the tricalcium phosphate and the subsequent biodegradation of tricalcium phosphate, ground sections of the excised skulls were prepared. A methylmethacrylate embedding technique was used. Due to the nature of tricalcium phosphate, ground sections cannot be prepared without embedding in a rigid fixation medium such as methyl methacrylate. Sections have been prepared both pre-stained with basic fuchsin and also unstained. The histology illustrated in this report has been stained with basic fuchsin.

Figure 10 demonstrates the bone-biomaterial interface in an animal (rabbit D-79) 3 months post implant. Note the bone formation generally following the longitudinal porosity from left to right across the slide. The non-connected porosity appears to be preferentially filled with connective tissue. Since the frequency of directional pores is less than desired, pores are only sporadically seen in the slide preparations.

Figure 11 demonstrates bone ingrowth into a 6-month sample (rabbit F-79). In this slide, two distinct longitudinal tracts can be seen coursing from left to right across the slide. Bone formation appears to be most common at the bone-biomaterial interface. The center of the directional tracts appear to contain connective tissue or be voids. Apparently, non-connected pores at the bottom of the slide are filled mostly with connective tissue.

~37X

FIGURE 10. Photomicrograph of bone-biomaterial interface in Rabbit D-79, 3 months post implant. Bone can be observed infiltrating progressively from the bone interface at the left, into the porosity of the implant. The longitudinal porosity is poorly defined in this slide. However, bone appears to be preferentially following the longitudinal pore.

This methacrylate embedded specimen was prestained in basic fuchsin, sectioned on a diamond cut-off wheel and hand polished to a thickness of approximately 75 microns. The same technique was used on all histology reported.

~37X

FIGURE 11. Photomicrograph of bone ingrowth into longitudinal pores of implant, six months post surgery (Rabbit F-79). Two distinct longitudinal pores can be observed extending from left to right across the slide. Bone formation appears most common at the interface of the bone-biomaterial, within the longitudinal pores.

Figure 12 illustrates an interesting pattern of bone ingrowth into directional pores at 9 months post implant (rabbit G-79). The upper directional pore, coursing from right to left across the figure is mostly filled with connective tissue; however, bone can be observed along the length of the pore in a thin layer adjacent to the ceramic. The small porosities radiating off the directional pore appear to be densely packed with bone.

Figure 13 is a slide from a 12-month animal (rabbit E-79). This sample has unfortunately been fragmented by the grinding process; consequently, the large clear void areas should be ignored when viewing the figure. A high percentage of the available area is filled with mature bone. Also, note there is relatively more bone than biomaterial present in the slide, thus indicating that a great deal of bioresorption has occurred. It is most important to note that there is no bone loss even at this late stage (12 months) of bioresorption. This implies that adequate bone formation has taken place to provide mechanical integrity to the area. This is in marked contrast to previous histologic results observed in omnidirectional samples. Generally, at 9 and 12 months post implant, omnidirectional samples showed total bone loss and connective tissue replacement in the area, due to loss of mechanical integrity.

No unidirectional samples in this report have exhibited bone loss up to 12 months. Apparently, the addition of the unidirectional pores has altered the mechanical situation adequately to allow the transition from biomaterial mechanical support of the area, to bone mechanical support of the area without loss of integrity. This is the most significant finding of the study.

Quantitative Histologic Analysis

The differences in ingrowth between the various samples were measured by computer digitization of histologic specimens. A Summagraphics® digitizer connected to a PDPLSI-11 microcomputer was used. An image of the histologic specimen was projected on the digitizer board. Two distinct areas of each implant in each animal were analyzed: one area was in the center of the implant and the other near the implant-bone interface; one implant was

~37X

FIGURE 12. Photomicrograph of implant 9 months post surgery, (Rabbit G-79). In this figure the bone interface is to the right. The center of the pore is filled with connective tissue or voids. Bone growth appears mostly at the biomaterial interface or in the small porosities radiating from the longitudinal pore.

~37X

FIGURE 13. Photomicrograph of implant one year post surgery (Rabbit E-79). This figure illustrates an area within the center of the implant. A high percentage of densely packed bone fills most available space within the biomaterial. The clear spaces are artifacts caused by fragmentation during the grinding process. It is important to note that a high percentage of the biomaterial has been replaced by bone, without total bone resorption of the area. This observation is a significant advance in the development of a bioresorbable bone scaffold material.

measured parallel to the pores (longitudinal) and the other segment at right angles to the pores (transverse). A standardized area of one square millimeter was measured from each sample analyzed.

The following parameters were calculated:

- percent total porosity (what percent of the observed area is porous),
- percent of pores filled with connective tissue,
- percent of pores filled with bone,
- total percent of pores filled (connective tissue plus bone in pores).

Table 1 is a compilation of these percentages for all animals in this study group.

Four vertical columns represent either edge of implant analysis for each sample or center of implant analysis for each sample. Two numbers are present in each data space separated by a slash (/). Data to the left of the slash is from the right half of the left implant, which was sectioned parallel to the long axis of the pores (longitudinal) and stained with basic fushsin. Data to the right of the slash (/) is from the front half of the right implant, which was sectioned across the pores (transverse) and stained with basic fuchsin.

There are several observations that can be made from this quantitative effort. Most important is that there was no bone loss noted. Unfortunately, there is no clear cut pattern to the data; however, all available space was filled with either bone or connective tissue with a consistently higher percentage of bone than seen previously.

The wide fluctuations in total porosity indicates the lack of uniformity in the porous material. Filling of available space with connective tissue and bone appears to be a regional phenomena. Generally, the filling of voids with bone is better than with previous omnidirectional samples analyzed in this manner. These results were probably weakened by the small sample size used.

TABLE 1. QUANTITATIVE HISTOLOGIC ANALYSIS OF TRICALCIUM PHOSPHATE. THE RESULTS WERE OBTAINED BY DIGITIZATION OF THE INDICATED STRUCTURES. ALL VALUES ARE DESCRIBED IN PERCENT. POROSITY IS PERCENT OF THE OBSERVED AREA. C. T. (CONNECTIVE TISSUE) IS PERCENT OF THE AVAILABLE PORES. BONE (B) IS DESCRIBED AS PERCENT OF AVAILABLE PORES AS IS THE TOTAL OF CONNECTIVE TISSUE AND BONE.

		Right Half of Left Implant Longitudinal Section / Edge of Sample Analysis (percent)				Front Half of Right Implant Transverse Section (percent) Center of Sample Analysis			
Rabbit #	Insertion Time	Total Porosity	Connective Tissue in Pores	Bone in Pores	CT and B in Pores	Total Porosity	Connective Tissue in in Pores	Bone in Pores	Connective Tissue and Bone in Pores
A-79	3 mo.	44.0/38.0	54.1/34.7	54.1/34.7	100.0/90.5	67.6/31.9	51.5/40.8	45.7/40.9	97.3/81.7
D-79	3 mo.	39.7/31.5	29.5/84.8	70.5/15.2	100.0/100.0	41.2/17.1	30.0/58.5	70.0/16.0	100.0/74.4
F-79 (No T cut)	6 mo.	50.8/	36.0/	64.0/	100.0/	47.3/	47.5/	52.5/	100.0/
G-79 (slides of L cut dark)	9 mo.	40.9/55.2	38.7/52.0	61.3/48.0	100.0/100.0	13.0/28.8	45.4/79.1	54.6/20.9	100.0/100.0
H-79 (slides of L cut dark)	9 mo.	25.5/46.9	86.4/28.8	13.6/71.2	100.0/100.0	24.5/29.6	19.6/14.8	80.4/85.2	100.0/100.0
C-79	12 mo.	23.8/43.7	54.5/41.7	45.5/58.3	100.0/100.0	26.9/34.3	54.9/69.6	45.1/30.4	100.0/100.0
E-79	12 mo.	65.5/49.1	36.7/33.6	60.5/66.4	97.2/100.0	62.3/44.4	36.2/47.1	63.8/52.9	100.0/100.0

CONCLUSIONS AND DISCUSSION

This study indicates that the use of longitudinally organized unidirectional pores is a viable method to prevent the deleterious loss of implant and bone integrity seen in previous experiments with omnidirectional specimens. This loss of integrity has continually plagued the use of structural bioresorbable bone scaffold materials. The classical approach to bioresorbable structural materials was to construct them with a uniform omnidirectional porosity. Unfortunately, this design has two major drawbacks. First, the strength of the material is severely limited. Second, and more importantly, as bone grows in and the material simultaneously biodegrades, mechanical integrity of the biomaterial-bone complex is usually lost. The result is a bone loss and filling of the area with connective tissue in response to loss of stability. The use of relatively large channels for bone to grow through appears to provide adequate mechanical integrity from the bone component through the implant composite to prevent bone loss.

The formation of unidirectional tricalcium phosphate has proven to be a much more difficult task than anticipated. Consequently, the resulting pilot material used in this study was of lower strength and much lower longitudinal pore density than desired; however, the material was adequate to demonstrate the concept.

Observation of the histologic samples provided the most convincing evidence of ingrowth without loss of integrity. The 12 month samples exhibited large volumes of mature bone throughout the samples with no indication of bone loss. (Generally, formation was favored in areas where the porosity was interconnected.) These histologic samples demonstrated the inconsistency of the material. This inconsistency confounded the quantitative analysis of the histologic samples. The quantitation indicated much less difference between samples collected at various time periods than was qualitatively obvious to the observer. The small sample size in this proof of concept experiment additionally weakened the quantitative analysis. Nevertheless, this analysis corroborated that no bone loss was visible in any of the samples. It should be recalled that our previous report(s) indicated that the same material without directed porosity demonstrated bone loss in <u>all</u>

9 and 12 month samples. This fact supports the hypothesis that directional porosity is a viable method of producing structural bioresorbable ceramic for bone scaffolding.

Sequential radiographic analysis adequately demonstrated resorption of individual samples. Again, as with histologic analysis, inconsistency of bioresorption was noted. Certain samples appeared to resorb at a much greater rate. The most probable cause for less than satisfactory resorption of certain samples is lack of porosity in certain regions. Obviously, a more consistent material is desirable.

The general conclusions of this study have been corroborated by Tortorelli[10] in research involving implantation of the same material in the dog mandible.

RECOMMENDATIONS

Since the concept of directional porosity appears viable, future efforts should be directed towards producing a higher quality material with unidirectional pores. This material should contain pores close to 400 microns in diameter. These pores should be spaced close together. The density of these pores should be determined by the mechanical strength of structural scaffold required. To that end, density of the tricalcium phosphate surrounding the directional pores should be increased. The material should have multiple layers of directed pores, interconnected by auxillary channels. A practical method of producing the desired material in large amounts should also be considered. When a higher quality directional material is available, the rabbit experiments as communciated in this report, as well as the dog mandible experiments performed at USAIDR, should be repeated for purposes of comparison.

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